

## WHAT IS CLAIMED IS:

1. A method for analyzing a complex protein mixture, said method comprising:

5 labeling one or more active target proteins present in said complex protein mixture by combining at least one probe, said probe(s) comprising a functional group specific for one or more active target proteins and a fluorescent moiety, with said complex protein mixture under conditions whereby said probe(s) react with said active target proteins; and

10 detecting a signal from one or more labeled active target proteins present in said complex protein mixture, wherein said signal is detected by separating one or more of said labeled active target proteins and generating a fluorescent signal from or more of said labeled active target proteins during or following said separation.

15 2. A method according to Claim 1, wherein said separation comprises applying all or a portion of said labeled active target proteins to an electrophoretic medium for separation of said labeled active target proteins; and

generating a fluorescent signal from one or more separated active target proteins, whereby said fluorescent signal indicates the presence of an active target protein in said complex protein mixture reactive with said at least one probe.

20 3. A method according to Claim 2, wherein said separation comprises SDS-PAGE.

4. A method according to Claim 2, wherein said separation comprises capillary electrophoresis.

25 5. A method according to Claim 1, wherein said functional group is selected from the group consisting of an alkylating functionality, an acylating functionality, a ketone functionality, an epoxide functionality an aldehyde functionality, a sulphonyl functionality and a phosphoryl functionality.

6. A method according to Claim 2, further comprising:

isolating at least one fluorescent band from said electrophoretic medium; and

identifying one or more labeled active target proteins present in said fluorescent band.

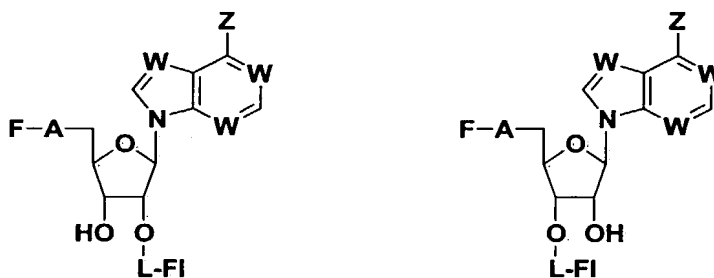
5 7. A method according to Claim 6, wherein one or more labeled active target proteins are isolated prior to generating said signal by binding to a receptor bound to a solid phase, wherein said receptor binds the probe labeling said active target proteins, removing unbound proteins, and releasing bound labeled active target proteins from said receptor.

10 8. A method according to Claim 1, wherein said fluorescent moiety exhibits a peak absorbance wavelength in the visible spectrum, and exhibits an peak emission wavelength in the visible spectrum

9. A method according to Claim 1, wherein said fluorescent moiety is a rhodamine.

15 10. A method according to Claim 7, wherein said rhodamine is 5-carboxytetramethylrhodamine or 6- carboxytetramethylrhodamine.

11. A method according to Claim 1, wherein said probe has the structure:



20 wherein:

each W is carbon or nitrogen;

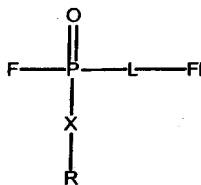
Z is hydrogen or amino;

F is a functional group capable of reacting with at least one of thiol, hydroxyl or amino joined through A to the 5' carbon of the ribose, where the functional group may be directly bonded to A or through a link, the functional group being a one or more moieties comprising, halogen, O, S, N, P, and C, selected from the group  
 5 consisting of fluorosulfonyl, fluorophosphonyl ester, halogen, epoxide, ethylene  $\alpha$  to an activating group, and halogen  $\beta$  to an activating group;

A is NR, O, S or CH<sub>2</sub>, wherein R is H or alkyl of from 1 to 6 carbon atoms;

Fl is a fluorescent moiety joined to the oxygen of the 2' and/or 3' position of the ribose through a linker moiety L of at least 2 atoms that comprises carbon,  
 10 oxygen, nitrogen and sulfur.

12. A method according to Claim 1, wherein said probe has the structure:



wherein

15 X is -CH<sub>2</sub>-, -O-, or -S-;

R is -H or a chain of from 1-20 carbon atoms and from 0 to 5 heteroatoms, which chain is straight or branched alkyl, alkenyl, or alkynyl optionally comprising one or more aromatic, alicyclic, heteroaromatic, or heterocyclic groups;

L is a linker moiety comprising from about 2 to 20 carbon atoms and having  
 20 from 0 to 10 heteroatoms, wherein L is aliphatic, alicyclic, aromatic or heterocyclic;  
 and

Fl is a fluorescent moiety

13. A method according to Claim 1, wherein said complex protein  
 25 mixture is a proteome.

14. A method for analyzing a proteome, said method comprising:

combining at least one probe, said probe(s) comprising a functional group specific for one or more active target proteins and a fluorescent moiety, with a first portion of said proteome, and combining said at least one probe with an inactivated second portion of said proteome, under conditions for reaction of said probe(s) with said active target proteins, to provide a first and second product mixture, respectively;

separating labeled active target proteins present in said first and second product mixtures;

generating a fluorescent signal from or more of said labeled active target proteins present in said first and second product mixtures during or following said separation.

15. A method according to Claim 14, wherein probes having different fluorescent moieties are employed in said first and second product mixtures.

16. A method according to Claim 14, wherein said first and second mixtures are applied to the same lane of said electrophoretic apparatus

17. A method according to Claim 14, wherein said separation comprises SDS-PAGE.

18. A method according to Claim 14, wherein said separation comprises capillary electrophoresis.

19. A method according to Claim 14, wherein said second portion of said proteome is inactivated by a noncovalent agent.

20. A method according to Claim 14, wherein said functional group is selected from the group consisting of an alkylating functionality, an acylating functionality, a ketone functionality, an epoxide functionality an aldehyde functionality, a sulphonyl functionality and a phosphoryl functionality.

21. A method according to Claim 14, further comprising:

isolating at least one labeled active target protein from said first product mixture.

22. A method according to Claim 21, wherein said isolating step  
5 comprises binding at least one labeled active target to a receptor bound to a solid phase, wherein said receptor binds the probe labeling said active target protein(s), removing unbound proteins, and releasing bound labeled active target proteins from said receptor.

23. A method for analyzing a proteome, said method comprising:

10 combining at least one probe, said probe(s) comprising a functional group specific for one or more active target proteins and a fluorescent moiety, with a first portion of said proteome, and with an inactivated second portion of said proteome, under conditions for reaction of said probe(s) with said target proteins to provide a first and second product mixture, respectively;

15 separating at least said first product mixtures by binding said one or more of said probe(s) to one or more receptors that specifically bind said probe(s), washing away unbound components of said at least first product mixture, and releasing bound components of said at least first product mixture;

20 applying said released components of said first mixture and the remaining portion of said second product mixture to an electrophoretic apparatus for separation of said product mixtures into individual bands;

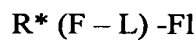
25 scanning said separation for fluorescent bands, whereby bands present in said first product mixture and at least 2-fold less in said second product mixture indicate the presence of active proteins in said proteome reactive with said at least one probe; and

proteolytically degrading active proteins to fragments and determining the sequence of at least one fragment bound to a probe.

24. A method according to Claim 23, wherein said second portion of said proteome is inactivated by a noncovalent agent.

25. A method according to Claim 24, wherein said determining comprises mass spectrometry.

5 26. A method for screening for molecules having an affinity for an active protein in a complex protein mixture from a biological source, employing a combinatorial chemical library comprising a plurality of members of the formula



wherein:

10 FI is a fluorescent moiety;

L is a linker moiety;

F is a functional group reactive at an active site of an active target protein; and

R is an affinity moiety of less than 1kDal;

15 wherein the \* intends that R is independently optionally present and R, if present, is bound to F, L or F and L,

said method comprising:

20 (a) combining with said complex protein mixture, in a native portion and an inactivated portion, one or more members of said combinatorial chemical library, under conditions for reaction of said member(s) with active target proteins to form conjugates in said native and inactivated portions;

(b) applying all or a portion of said native and inactivated portions to an electrophoretic medium for separation of said conjugates into individual bands; and

(c) comparing a fluorescent signal obtained from corresponding bands present from said native and inactivated portions;

whereby bands in said native portion that provide a fluorescent signal that is at least 2-fold greater than a fluorescent signal provided by a corresponding band in said inactivated portion are comprised of active proteins reactive with members of said combinatorial library.

5           27.    A method according to Claim 26, wherein all or a portion of said native and inactivated portions are applied to the same lane in said electrophoretic medium.

          28.    A method according to Claim 27, wherein said fluorescent moieties are independently selected from the group consisting of xanthene dyes,  
10   naphthylamine dyes, coumarins, cyanine dyes and metal chelate dyes, BODIPY, lanthanide cryptates, and erbium, terbium and ruthenium chelates.

          29.    A method according to Claim 28, wherein said fluorescent moieties in said first and second combinatorial libraries do not affect the relative mobilities of said conjugates in said gel electrophoresis.

15           30.    A method according to Claim 28, wherein said fluorescent moieties are cyanines.

          31.    A method according to Claim 28, wherein said fluorescent moieties are rhodamines.

          32.    A method according to Claim 31, wherein said rhodamines are 5-  
20   carboxytetramethylrhodamine or 6- carboxytetramethylrhodamine.

          33.    A method for screening for molecules having an affinity for an active protein in a complex protein mixture from a biological source, employing a combinatorial chemical library comprising a plurality of members of the formula

25    $R^* (F - L) - FI$

wherein:

FI is a fluorescent moiety;

L is a linker moiety;

F is a functional group reactive at an active site of an active target protein; and

R is an affinity moiety of less than 1kDal;

wherein the \* intends that R is independently optionally present and R, if present, is  
5 bound to F, L or F and L,

said method comprising:

(a) combining each member of said chemical library, individually or as a  
10 plurality of members, with all or a portion of said complex protein mixture under  
conditions whereby said member(s) react with active proteins present in said  
complex protein mixture to form covalent conjugates comprising one of said  
member(s) and one of said active proteins;

(b) separating one or more of said conjugates; and

(c) generating a fluorescent signal from one or more separated  
15 conjugates, whereby said fluorescent signal indicates the presence of an active target  
protein in said proteome reactive with at least one member of said chemical library.

34. A method according to Claim 33, wherein said separation comprises  
applying all or a portion of said labeled conjugates to an electrophoretic medium for  
separation of said labeled conjugates.

20 35. A method according to Claim 34, wherein said separation comprises  
SDS-PAGE.

36. A method according to Claim 34, wherein said separation comprises  
capillary electrophoresis.